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2,4-DICHLOROPHENOXYACETIC ACID AND
ITS CALCIUM SALT FROM SIX DIFF-
FERENT SOILS BY LEACHING

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
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PHILOSOPHY

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REMOVAL OF 2,4-DICHLOROPHENOXYACETIC ACID AND ITS CALCIUM SALT FROM SIX DIFFERENT SOILS BY LEACHING¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 578

ROBERT W. HANKS

Introduction

Most reports to date on the use of 2,4-dichlorophenoxyacetic acid (2,4-D) as a herbicide deal with spray application to vegetation. The selective nature of the substance has been well demonstrated. The direct treatment of soils with 2,4-D in order to kill weed seeds and for other purposes is chiefly in the experimental stage. Published results of such experiments suggest that the length of persistence of the toxic effect is affected by soil type and management practice. There seems to be considerable variation in persistence of 2,4-D in soils: from less than 4 weeks in muck (3) to as much as 18 months in soil stored in a relatively dry condition (5). The response of clover indicated a difference in the persistence of the acid in Rothamsted clay and Woburn sand (6). KRIES (4) found a longer period of toxicity in soil to which lime had been added a few days prior to application of the acid than in unlimed soil; she noted, however, that additions of partially decomposed leaf mold at the

time of liming tended to reduce the degree and persistence of toxicity in the limed soil. BALL (1) reported that toxic effects of the acid were more pronounced in one of three soils tested than in the others, and for a longer period of time. 2,4-Dichlorophenoxyacetic acid was more readily leached from soil than were other herbicides (2), although it is still unknown whether the disappearance of the toxic effect is due chiefly to leaching, to biological decomposition, to a chemical or a physical combination with components of the soil, or to all these causes.

The purpose of the experiment here reported was to determine the relative rates at which 2,4-dichlorophenoxyacetic acid and its calcium salt could be leached from soils possessing different physical and chemical properties. The calcium salt was used in order to determine if it would be more persistent or less readily leached than the acid; this possibility was indicated by KRIES's results (4) on limed soils. Acid and alkaline soils were tested in order to investigate further a possible relationship between soil reaction and persistence or degree of toxicity of the herbicides.

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Methods

The sources and some properties of the six soils which were tested are listed in table 1. Soil pH was determined with the glass electrode, moisture equivalent by the centrifuge method.

Each soil lot was thoroughly air-dried and passed through a $\frac{1}{4}$ -inch screen in order to obtain a uniform maximum size of soil particles. A small aliquot of each soil was then removed to serve as a control, receiving no chemical treatment. With the exception of soil F, half of each soil was treated with lime (CaCO_3) at a rate approximately equivalent to 2000 pounds per acre to a 6-inch depth. Be-

or chloroform. Each batch of treated sand was air-dried and mixed to insure uniformity of this material used in treating the soils.

The sand-acid mixture and the sand-salt mixture were applied to the soils as follows: half of the limed and half of the nonlimed aliquots of each soil were treated with the sand-acid mixture to supply 20 p.p.m. by weight of the acid in the soil; the remaining aliquots of limed and nonlimed soils were treated with the sand-salt mixture to give approximately 22.3 p.p.m. by weight of the calcium salt in the soil. Thus there was applied in equal weights that part of

TABLE 1

Soil	Source	Physical characterization	pH	Moisture equivalent
A.....	Beltsville, Md.	Sandy	3.8	10.99
B.....	Lake Geneva, Wis.	Silt loam	7.7	15.57
C.....	Lake Geneva, Wis.	Peat	6.7	135.04
D.....	Chicago, Ill.	Clay loam	8.4	28.55
E.....	Gary, Ind.	Sandy loam	7.1	15.23
F.....	Hurley, N.M.	Sandy clay	8.4	25.50

cause it was naturally alkaline, only a small aliquot of soil F was limed. The limed aliquots were placed in flats in the greenhouse and were kept moist (not leached) for 2 weeks to permit the reaction of the calcium with other constituents of the soil to approach equilibrium.

In order to obtain a uniform distribution of 2,4-D and its calcium salt within the soils, sand-herbicide mixtures were prepared quantitatively by pouring an ethyl alcohol solution of the acid and an ethyl alcohol mixture of the salt over pure quartz sand. It was necessary to use the alcoholic mixture of the calcium salt rather than a solution because the salt is insoluble in water, ethyl alcohol, diethyl ether, petroleum ether, acetone,

the molecule of these herbicides which is considered to be responsible for their toxic effects (8). Thus, four conditions were obtained for each soil type: (1) lime with the acid, (2) lime with the calcium salt, (3) the acid without lime, and (4) the calcium salt without lime.

After incorporating the sand-herbicide mixtures with the soils, 4-inch glazed crocks with side-hole drains were filled to a depth of approximately 6 inches. Each drain had been covered with glass wool and fitted with a cork stopper and glass tube to facilitate collection of the leachate in Erlenmeyer flasks. There was a minimum of three pots of soil for each treatment of soil A; five or six pots were available for each treatment of the other five soil types.

The potted soils were placed in a basement laboratory in order that water loss by evaporation might be reduced to a minimum and to provide uniform temperature conditions. All pots were wetted initially, with the drains closed, with the equivalent of 4 surface-inches of water. This permitted each soil mass to become thoroughly wetted before any leachate was collected. In addition to the initial 4 surface-inches of water, one-half of the pots in each soil treatment received the equivalent of 10 surface-inches of water over a 6-week period; the other half received the equivalent of 16 surface-inches in the same period. The 10 surface-inches of water were applied at the rate of 1 inch per week for the first 2 weeks and 2 inches per week thereafter. The 16 surface-inches were added at the rate of 2 inches per week for the first 2 weeks and 3 inches per week thereafter. The temperature in the laboratory was 26° C. throughout the experiment.

The leachate from each soil treatment was tested once a week for the presence of herbicide by a slight modification of the bio-assay developed by SWANSON (7). In his method SWANSON used hybrid field corn of the Silver King (Wisconsin no. 7) variety germinated for 48 hours in 6-inch Petri dishes. Twelve to fifteen seedlings with roots between 15 and 25 mm. in length were then placed on filter paper in other 6-inch Petri dishes and moistened with 15 ml. of solution containing 2,4-dichlorophenoxyacetic acid. After 48 hours the increase in average root length of the seedlings in each dish was determined and expressed as a percentage of the increase in average root length of control seedlings treated with distilled water. All germination and growth were in darkness at 26° C. SWANSON determined concentrations of the

acid ranging from 1/100 p.p.m. to 2 p.p.m. with only a 5% error.

The bio-assay used in the writer's experiment measured the relative toxicity of the leachates rather than the absolute concentration of the herbicide for which the test was devised originally. SWANSON's method was modified by use of 9-cm. Petri dishes, ten seedlings to the dish treated with 10 ml. of leachate. All other conditions of the original method were unchanged.

Results and discussion

The results of the weekly bio-assays on leachates from soil samples leached with the equivalent of 10 and 16 surface-inches of water showed similar trends within each soil type. Therefore, only the results obtained from the application of the larger amount are reported graphically (fig. 1). The increase in average root length of seedlings grown in leachate from each soil treatment is expressed as a percentage of the increase in root length of seedlings grown in the leachate from untreated control soil of the same type. Each bar of the graph shows this percentage value obtained from the bio-assay of a single leachate. A block of six bars summarizes the results from a single soil treatment, each bar of a block, from left to right, representing the results of each successive week of the 6-week period. Percentage values much greater than 100 may be considered to indicate a stimulation in growth rate caused by the effects of the herbicide; those much below 100 show the relative degree of toxicity of the various leachates.

The results of the bio-assays indicate no significant or consistent difference in the degree of toxicity, in comparable leachates, of the acid and of the calcium salt at the concentrations used. The cal-

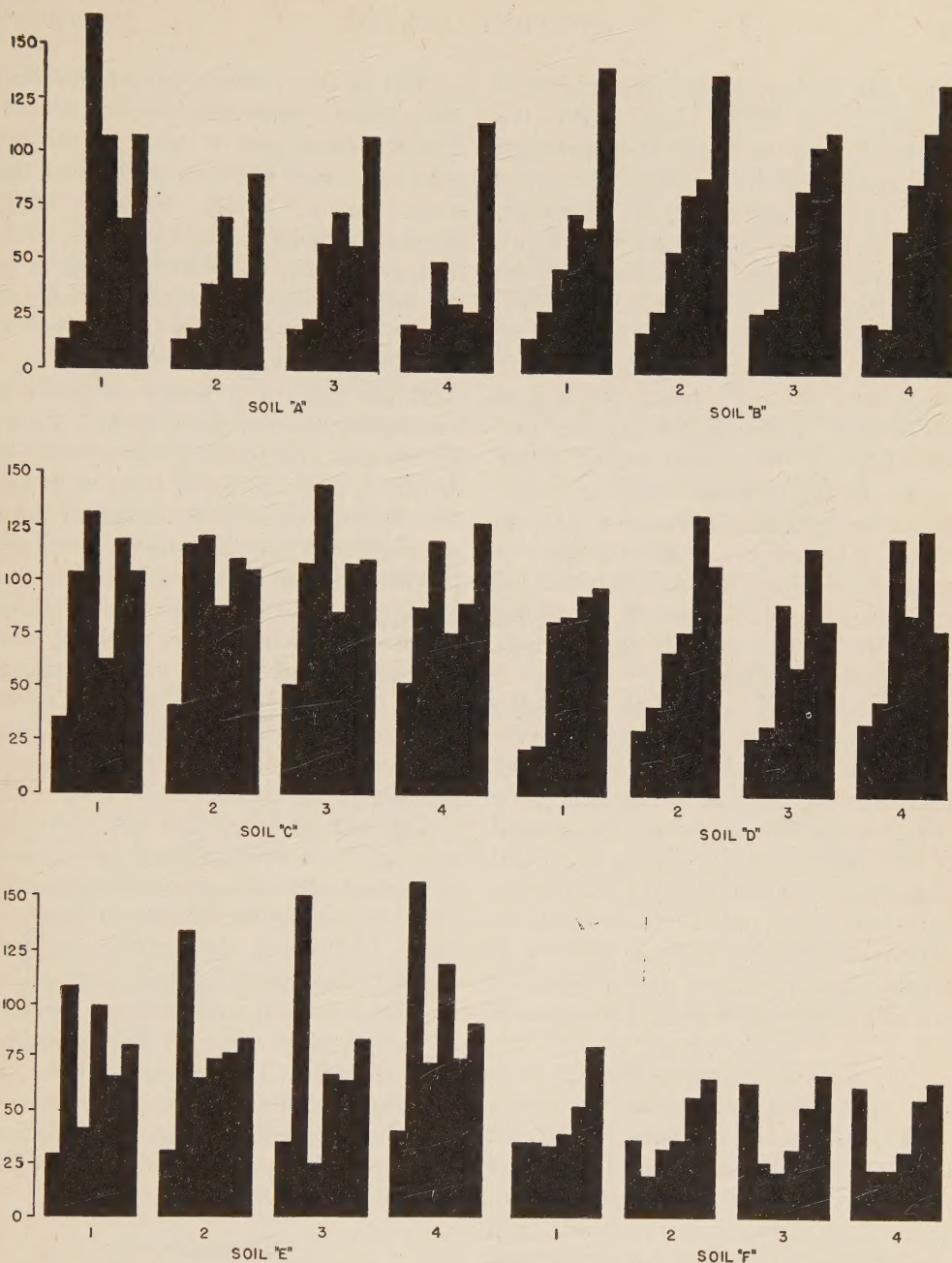


FIG. 1.—Percentage of corn-root elongation in leachate from each soil treatment based on its own control, indicating extent and rate of loss of herbicidal effect. Each bar of a block gives percentage value from bio-assay on single leachate. A block of six bars summarizes results from a single soil treatment, each bar from left to right representing results of each successive week of the 6-week period. Numbers below each block designate soil treatments: 1, limed soil with 2,4-dichlorophenoxyacetic acid; 2, limed soil with calcium salt of 2,4-D; 3, nonlimed soil with 2,4-D; 4, nonlimed soil with calcium salt.

cium salt, or its reaction products, thus seems to be as soluble as the acid in the soil solution of the tested soils, although the calcium salt is insoluble in water, ethyl alcohol, diethyl ether, petroleum ether, acetone, or chloroform. In general, the addition of lime to the soils likewise seemed to produce no significant differences in toxicity of leachates. In contrast, KRIES (4) found a prolongation of toxic effects of 2,4-D in soil in the presence of added lime, although the soil she used was different from those tested by the writer. It may be, also, that the nature of the leachate from soil is such that the degree of sensitivity in the bio-assay is reduced. However, the toxic effects of the herbicides persisted longer in one of the naturally alkaline soils (F) than in any of the other soils tested.

The bio-assay indicated that the leachate from soil C (peat) was relatively free of toxicity at the end of 2 weeks. HAMNER *et al.* (3) reported that the toxic effects of 2,4-D in muck soil disappeared in less than 4 weeks; KRIES (4) found that organic matter added to soil treated with both lime and 2,4-D reduced the period of toxicity. Thus it seems that organic matter in some way reduces the period of toxicity of these herbicides in soil.

The cause of the great fluctuation in the results obtained during the first 3 weeks with soil E is not known. With the fluctuation present in all four treatments of this soil, some unknown, and therefore uncontrolled, factor may have been operating in the control samples of that soil.

The relative toxicity in the successive leachates from soil B decreased at a more uniform rate than in the leachates from the other soils. Soil C was apparently rendered relatively free of toxicity with as little as 4 surface-inches of water

applied over a 2-week period, whereas toxic effects were still present in the leachate from soil F after treatment with four times as much water over the 6-week period. Besides leaching, other factors, such as biological decomposition, adsorption by colloidal systems in the soil, and chemical reactions forming nontoxic compounds may be involved in reducing toxicity.

At the end of 6 weeks of leaching treatment, all pots were moved to the greenhouse, and the soil was allowed to dry for 5 days. The soil from each pot was then mixed in such a manner that, when returned to the original pot, the portion that had been at the bottom was uppermost. Thus, any herbicide which had not been removed by leaching from the bottom layers of soil was immediately in contact with germinating seeds. Each pot was planted with ten red kidney beans to determine any residual effects of the herbicide.

This test indicated that soil F was the only soil from which all toxic effects due to the herbicide had not yet disappeared, thus confirming the results of the bio-assay. In this soil, the toxic effects were found to be greater in those pots which had been leached with only 10 surface-inches of water than in those leached with 16 inches. The toxicity did not affect the percentage of germination and was shown chiefly in the malformation and mottled appearance of the primary and first trifoliate leaves.

Summary

1. Six soils with a wide range in pH and in physical properties were treated with lime, 2,4-dichlorophenoxyacetic acid, and the calcium salt of the acid in such a manner as to obtain four conditions for each soil.
2. The treated soils were leached with

water at rates equivalent to 10 and 16 surface-inches over a 6-week period. The leachates were collected and tested weekly with a corn-seed bio-assay to determine relative toxicities.

3. The bio-assays indicated that the leachates from soil C (peat) were relatively nontoxic at the end of 2 weeks and that those from all soils except soil F (naturally alkaline) were relatively free of the herbicides at the end of 6 weeks. There were no significant differences in the degree of toxicity and its persistence in limed as compared with unlimed samples within each soil type or in that of the calcium salt versus the acid.

4. At the end of 6 weeks, the bottom

half of each soil mass was tested for residual toxic effects by the germination of red kidney beans. This test indicated that soil F was still somewhat toxic from the treatment and that the samples of this soil leached with 10 surface-inches of water were more toxic than those leached with 16 surface-inches of water. All other soils were then free of the toxic effect of the herbicide.

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